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Correction

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Correction for “Predictable allele frequency changes due to habitat fragmentation in the Glanville fritillary butterfly,” by Toby Fountain, Marko Nieminen, Jukka Sirén, Swee Chong Wong, and Ilkka Hanski, which appeared in issue 10, March 8, 2016, of *Proc Natl Acad Sci USA* (113:2678–2683; first published February 22, 2016; 10.1073/pnas.1600951113).

The authors note that Rainer Lehtonen should be added to the author list between Swee Chong Wong and Ilkka Hanski. Rainer Lehtonen should be credited with designing research and analyzing data. The corrected author line, affiliation line, and author contributions appear below. The online version has been corrected.

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Predictable allele frequency changes due to habitat fragmentation in the Glanville fritillary butterfly

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Contributed by Ilkka Hanski, January 22, 2016 (sent for review June 15, 2015; reviewed by Andrew P. Hendry)

Describing the evolutionary dynamics of now extinct populations is challenging, as their genetic composition before extinction is generally unknown. The Glanville fritillary butterfly has a large extant metapopulation in the Åland Islands in Finland, but declined to extinction in the nearby fragmented southwestern (SW) Finnish archipelago in the 20th century. We genotyped museum samples for 222 SNPs across the genome, including SNPs from candidate genes and neutral regions. SW Finnish populations had significantly reduced genetic diversity before extinction, and their allele frequencies gradually diverged from those in contemporary Åland populations over 80 y. We identified 15 outlier loci among candidate SNPs, mostly related to flight, in which allele frequencies have changed more than the neutral expectation. At outlier loci, allele frequencies in SW Finland shifted in the same direction as newly established populations deviated from old local populations in contemporary Åland. Moreover, outlier allele frequencies in SW Finland resemble those in fragmented landscapes as opposed to continuous landscapes in the Baltic region. These results indicate selection for genotypes associated with good colonization capacity in the highly fragmented landscape before the extinction of the populations. Evolutionary response to habitat fragmentation may have enhanced the viability of the populations, but it did not save the species from regional extinction in the face of severe habitat loss and fragmentation. These results highlight a potentially common situation in changing environments: evolutionary changes are not strong enough to fully compensate for the direct adverse effects of environmental change and thereby rescue populations from extinction.

adaptive genetic response | contemporary evolution | evolution of dispersal | global change | historical DNA samples

Changes in land use have greatly reduced the abundance and caused the extinction of innumerable populations and of many species (1–5). The remaining isolated remnant populations are expected (6, 7) and observed (8–10) to lose genetic variation via drift and inbreeding (with a few notable exceptions, e.g., ref. 11), which may further hasten their decline (12). Thus, gradual genetic deterioration along with demographic processes (13, 14) can contribute to the extinction debt in communities inhabiting fragmented landscapes. At the same time, increasing habitat fragmentation may strengthen selection on traits that reduce the risk of extinction of local populations and increase the rate of establishment of new populations to compensate for local extinctions (15). For example, species occupying recently fragmented habitats may evolve an increased (16) or decreased (17, 18) dispersal capacity, depending on factors such as the opportunity for successful colonization, the cost of dispersal, and the degree of temporal demographic variation (19). Although several studies have investigated the genetic composition of declining populations using neutral markers [e.g., Florida panthers (20) and greater prairie chickens (21)], there is a more limited understanding of the adaptive evolutionary dynamics of populations along the trajectory to extinction (although see ref. 22).

European butterflies have been greatly affected by land use changes, especially by changing agricultural practices and habitat fragmentation (23). The Glanville fritillary butterfly (*Melitaea cinxia*) has greatly declined in western and northern Europe. In Finland, the species went extinct from the mainland and southwestern (SW) archipelago in the 1970s (24), and it is now

restricted to the Åland Islands (Fig. 1), where it has a large metapopulation in a network of 4,000 dry meadows (16, 25). Although the extinction risk of small local populations is increased by inbreeding (26), along with many demographic processes, the metapopulation as a whole has shown no declining trend for the past 25 y (25), although the amplitude of fluctuations in the metapopulation as a whole has increased, probably due to climate change (27). In contrast, an isolated population on a small island, Pikku Tytärsaari, in the northern Baltic, shows great loss of genetic diversity and reduction of fitness due to accumulation of genetic load (9).

There is both phenotypic and genetic evidence demonstrating that the Glanville fritillary has the capacity to adapt to fragmented habitat. Butterflies inhabiting fragmented landscapes, including the Åland Islands, have significantly higher flight metabolic rate than butterflies from continuous landscapes (28). Increased flight metabolic rate in turn enhances dispersal rate (29) and colonization rate (30) in fragmented landscapes, thereby facilitating long-term survival (31). In an RNA-seq study of gene expression, 1,841 genes were differentially expressed between fragmented and continuous landscapes. Genes that were systematically up-regulated following an experimental flight treatment had higher basal expression in fragmented than in continuous landscapes (32).

The above results raise questions about the populations that went extinct in SW Finland in the 20th century. Did they show reduced genetic variation that might have contributed to their extinction? How related were these populations to the large Åland metapopulation and was there increasing genetic divergence in the declining populations? Are there signs of selection in flight-related genes (or other genes) in the extinct populations that inhabited the highly fragmented landscape? Here, we genotype extinct and extant

Significance

Understanding how species respond to environmental changes is essential for comprehending their ecological and evolutionary dynamics. Changes in land use have led to widespread loss and fragmentation of many habitats, resulting in the extinction of innumerable populations and species. Here, using historical DNA samples from now extinct populations that used to live in a highly fragmented landscape, we demonstrate past natural selection for better colonizing capacity. We also found highly reduced genetic diversity in the isolated populations prior to their extinction. These results demonstrate that, although species may show adaptation to rapidly changing landscapes, the evolutionary changes may not be strong enough to offset the direct adverse effect of environmental change and thereby to rescue populations from extinction.

Author contributions: R.L. and I.H. designed research; T.F., M.N., and S.C.W. performed research; T.F., J.S., S.C.W., R.L., and I.H. analyzed data; and T.F., S.C.W., and I.H. wrote the paper. Reviewers included: A.P.H., McGill University.

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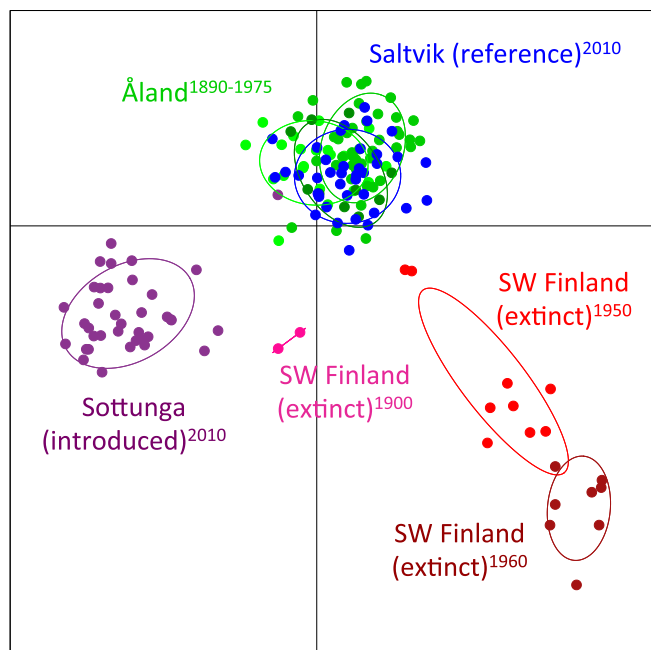


Fig. 2. DAPC ordination based on 222 SNPs with samples aggregated in space and time: Åland¹⁹⁰⁵ (1889–1921), Åland¹⁹⁴⁵ (1936–1949), and Åland¹⁹⁶⁵ (1957–1974) for museum samples from the Åland Islands and SW Finland¹⁹⁰⁰ (1880–1920), SW Finland¹⁹⁵⁰ (1945–1954), and SW Finland¹⁹⁶⁰ (1962–1968) for museum samples from the now extinct populations in SW Finland, and contemporary samples from the mainland Åland and the 24-y-old introduced metapopulation in Sottunga (see Fig. 1 for their locations). F_{ST} values calculated for the different groups show that the SW Finnish samples become increasingly differentiated from the Åland samples over time (SI Appendix, Table S1).

Finland, consistent with the results in Fig. 2. In the Åland samples, the neutral markers showed significantly greater F_{ST} than the SNPs in the candidate genes (*SI Appendix, Fig. S1*; LM $F = 15.8$, $P = 0.0001$), suggesting that some of the latter markers have been affected by stabilizing selection.

Systematic Allele Frequency Changes in Candidate Genes. We identified 10 outlier loci among the candidate genes ($n = 164$) in museum samples from Åland and 10 from SW Finnish populations (with all time periods pooled together), of which five were common to both datasets ([Dataset S2](#)). For the 34 neutral SNPs, the respective numbers were four and one ([Dataset S3](#)). Given that we tested 150 candidate SNPs, and assuming that the two datasets are independent, the expected number of outlier SNPs in both datasets is 0.6, conditional on the observed numbers in each dataset. The probability of having at least five shared outliers in the two sets is very low— $4.8e^{-05}$ (from the hypergeometric distribution). However, after correcting for multiple testing, no SNP remained significant. Using BayeScan, an alternative method, Mc1:2673:141336 was identified as a marginally significant outlier after correcting for multiple testing [false discovery rate (FDR) = 0.05] (it was significant without correction in the first analysis). Although the 15 outliers were not significant after correcting for multiple testing, they nonetheless represent the most divergent loci among the set of candidate SNPs. Moreover, lack of significance does not invalidate the analyses below, where we test possible correlations in allele frequencies in the extinct SW Finnish populations and in three other independent datasets (the results of these comparisons for each outlier locus are summarized in [SI Appendix, Table S3](#)). We excluded two SNPs that are located in the sex chromosome (Mc1:1791:57299 and Mc1:3568:15483) and are hence affected by varying sex ratio in the small samples, and one marker (Mc1:4593:27912), which is known to be associated with host plant preference (34) and is therefore affected by the varying

distribution of the two host plants across the study areas (no *V. spicata* in SW Finland).

The first analysis asks how repeatable the allele frequency changes are in response to habitat fragmentation. For this purpose, we compared the extinct SW Finnish populations with the 24-y-old introduced Sottunga metapopulation, which also inhabits a highly fragmented landscape, a small sparse network of 51 habitat patches. Allele frequencies in SW Finland and Sottunga have shifted mostly in the same direction from Saltvik frequencies (Fig. 3 and *SI Appendix*, Fig. S2; LM: $R^2 = 0.36$, $df = 11$, $P = 0.02$). There is no comparable agreement in allele frequency changes in neutral markers (*SI Appendix*, Figs. S3 and S4; LM: $R^2 = -0.004$, $df = 19$, $P = 0.35$). Importantly, allele frequency changes in the 24-y-old introduced Sottunga metapopulation have been significantly smaller than in the SW Finnish populations, which have had on average 80 y to diverge (Fig. 3; paired t test: $t = 2.59$, $df = 11$, $P = 0.03$). These results point to systematic changes in allele frequencies in the outlier loci in populations inhabiting fragmented landscapes, and hence the results suggest that nonrandom processes have influenced the changes.

Population Turnover and Allele Frequency Changes. The Sottunga metapopulation consists of, and the now extinct SW Finnish populations have consisted of, small local populations with frequent population turnover. Repeated recolonizations may have selected for particular genotypes. For example, individuals sampled from newly established populations have a high frequency of a genotype associated with high dispersal capacity (16, 35, 36). To test this possibility, we calculated the difference in allele frequencies of outlier loci in newly colonized versus old local populations in contemporary Åland samples and correlated this difference with changes in allele frequencies in the samples from Sottunga and SW Finland in relation to the pooled sample from old large populations in the contemporary Åland metapopulation. Fig. 4 shows that allele frequencies have generally shifted in the same direction as newly established populations differ from old local populations (SW Finland: LM, $R^2 = 0.36$, $df = 11$, $P = 0.02$; Sottunga: LM, $R^2 = 0.14$, $df = 11$, $P = 0.13$). These results suggest that dispersal and recolonization processes that lead to the allele frequency difference between new and old local populations have influenced allele frequency changes in the introduced Sottunga metapopulation.

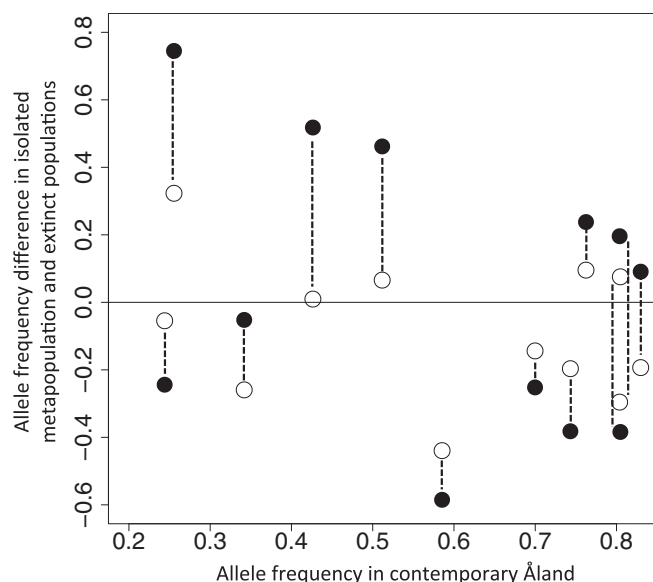


Fig. 3. Allele frequency differences of outlier loci ($n = 12$; excluding two loci on the sex chromosome and Mc1:4593:27912, which is known to be associated with host plant preference) between samples from now extinct populations in SW Finland (black) and the introduced metapopulation in Sottunga (white), compared with old, well-connected local populations in Saltvik.

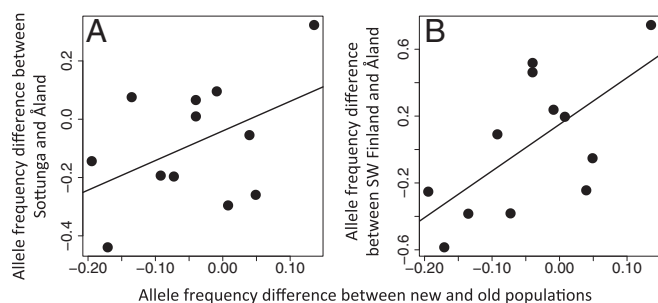


Fig. 4. Allele frequency changes in the outlier loci ($n = 12$) in (A) the introduced Sottunga metapopulation and (B) the extinct SW Finnish populations from the contemporary Saltvik reference (old local populations), plotted against the allele frequency difference between newly established versus old local populations in the contemporary metapopulation. Allele frequencies have shifted in the same direction in Sottunga and SW Finland as newly colonized populations differ from old local populations (Sottunga, $R^2 = 0.14$, $P = 0.13$; SW Finland, $R^2 = 0.36$, $P = 0.02$).

during its 24-y history and in the museum samples from the extinct SW Finnish populations between 1880 and 1968.

Allele Frequency Changes and Habitat Fragmentation. The above results suggest that allele frequencies of outlier loci in Sottunga and SW Finland have shifted in the direction expected to occur in highly fragmented landscapes with frequent local extinctions and recolonizations. To test this hypothesis directly, we compared the allele frequency changes of outlier loci in Sottunga and SW Finland with allele frequencies in four regional populations in the Baltic region (data from ref. 32; Fig. 1 shows their locations). Two regional populations, Åland in Finland and Uppland in Sweden, inhabit highly fragmented landscapes, whereas the two others, Saaremaa in Estonia and Öland in Sweden, occur in landscapes with much more continuous habitat (28). Three outliers had to be excluded because their allele frequencies could not be extracted from the RNA-seq dataset for all four regional populations. In a principal component analysis of outlier allele frequencies for these four regional populations, the second principal component (PC2, $R^2 = 0.33$) was strongly positively correlated with allele frequencies in fragmented populations and strongly negatively correlated with allele frequencies in the continuous populations (SI Appendix, Table S4). Large values of PC2 thus indicate a pattern of allele frequencies in fragmented landscapes. Allele frequencies in Sottunga and SW Finland have shifted in the direction of allele frequencies in fragmented landscapes (SI Appendix, Fig. S5; LM: $R^2 = 0.30$, $df = 8$, $P = 0.07$).

Discussion

To summarize the findings of this study, allele frequencies at 12 outlier loci, which have been under putative positive selection or linked to genes under selection, have shifted in the same direction in two independent datasets, a 24-y-old small introduced metapopulation on the island of Sottunga and museum material from now extinct populations in SW Finland during the 20th century. Both landscapes are highly fragmented, and we suggest that nonrandom processes related to rapid population turnover in highly fragmented landscapes have affected these allele frequency changes. The landscape in SW Finland is an archipelago, a naturally highly fragmented landscape, and hence the sparse and small butterfly populations in the 20th century likely exhibited much turnover before the eventual regional extinction. For Sottunga, which was introduced as an experiment 24 y ago, we have a complete record of demographic dynamics in the network of 51 small meadows. All local populations have gone extinct at least once during this period, and hence the very persistence of the metapopulation is definitely due to frequent recolonizations compensating for local extinctions; the current butterflies are the offspring of recent colonizers. The hypothesis that dispersal and recolonizations have affected the allele frequency changes is further supported by the result that these changes are consistent with allele frequency differences between newly

established versus old local populations in the contemporary metapopulation in Åland, which reflects the immediate effect of recolonizations over one generation. Moreover, the allele frequencies in the introduced Sottunga metapopulation and the extinct SW Finnish populations have shifted in the direction observed in highly fragmented landscapes elsewhere in northern Europe. In brief, the likely mechanism leading to these results is high population turnover in Sottunga and SW Finland having selected genotypes that enhance dispersal and recolonization, an adaptive genetic response to habitat fragmentation.

The 15 outlier loci (12 loci after filtering) were not significant following the correction for multiple testing. This is not surprising because our sample size is relatively small and hence the power of the statistical test is limited. However, we emphasize the significant and consistent patterns obtained while comparing the primary dataset from SW Finland with not just one but three other, completely independent datasets. These comparisons make the interpretation of the results much stronger than what would have been possible for significant outliers in a single outlier analysis.

Previous studies on the Glanville fritillary (16, 35, 36) and other species (37, 38) have demonstrated that colonizations select for more dispersive individuals than the average individual in the metapopulation. In the Glanville fritillary, female butterflies from newly established local populations (39, 40) and from highly fragmented landscapes (28) have a higher flight metabolic rate than those from old local populations and from continuous landscapes. Flight metabolic rate is positively correlated with dispersal rate in the field (29). The majority of the outlier loci in the present study have been implicated as being related to flight in previous studies, although the annotations are not informative (Dataset S2).

Selection for more dispersive butterflies in the declining populations in SW Finland in the 20th century may have made the metapopulation more likely to persist, although the species went extinct from this entire region in the 1970s, which has been attributed to very extensive habitat loss and fragmentation due to agricultural intensification (24). In contrast, the Åland metapopulation has survived because habitat loss and fragmentation have been limited due to topographical and cultural reasons (41). As we have seen in this study, there has been no measurable reduction in heterozygosity in Åland over the past century. In the SW Finnish archipelago, habitat loss and fragmentation were so extensive that no evolutionary response in dispersal rate could compensate for it, which is likely to be a common situation in changing environments (42). It is also possible that any beneficial effects from genes linked with the outlier loci were swamped by accumulation of deleterious mutations (6, 43, 44) and general loss of heterozygosity and fitness (45). In the Glanville fritillary, flight metabolic rate is significantly and positively correlated with genome-wide heterozygosity, a proxy of individual inbreeding (46), with a 30% fitness reduction in a small ($N_e \sim 100$) population that has remained completely isolated for at least 75 y (9). As another example, habitat fragmentation due to climate-induced range contraction has eroded genetic diversity in an alpine mammal species (47), likely reducing the adaptive potential of the species and increasing the risk of inbreeding (48).

Many previous studies have identified likely adaptive genetic responses to environmental change by studying species along environmental gradients (e.g., refs. 49–51). A complication with such studies is possibly the dissimilar evolutionary and demographic histories of populations along the gradient. A more direct approach is to monitor changes in molecular markers (e.g., refs. 52–54) or quantitative traits (e.g., refs. 55–58) across time, although in most cases the time span that has been covered is relatively short. For example, a study of *Drosophila melanogaster* showed significant shifts in allele frequencies along a latitudinal cline over a 20-y period, consistent with increasing temperatures (52). Hansen et al. (42) proposed six criteria that are needed to demonstrate adaptive genetic responses to environmental change. The present study has fulfilled all these criteria: (i) Suitable genetic variation for selection to act upon, which is evidently the case here. (ii) This genetic variation is linked to a specific environmental stress; our SNP panel was selected to include especially markers associated with either flight or

habitat fragmentation (32). (iii) Any identified associated alleles vary across generations, which is the case here. Moreover, we have shown that allele frequency changes have become greater with increasing length of time (Fig. 3). (iv) Allele frequency changes reflect selection, which was demonstrated by comparing allele frequency changes in candidate loci with changes in neutral markers. (v) The observed allele frequency changes are attributable to the identified environmental stress, which in our case is the process of population turnover in highly fragmented landscapes. Finally, (vi) any changes in allele frequency cannot be attributed to a new well-colonizing population simply replacing the original population, which is most directly known for the introduced metapopulation on the island of Sottunga, continuously monitored since its establishment in 1991.

In conclusion, to our knowledge, this study is the first to demonstrate predictable changes in allele frequency in response to habitat fragmentation. This study also highlights a likely common situation in changing environments, where adaptive genetic responses may not be strong enough to rescue populations from extinction due to adverse effects of rapid environmental change.

Materials and Methods

Museum Samples. We searched museum collections for specimens collected across the entire historical range of the species in Finland. Altogether, 119 specimens were located, originating from 11 regions in the Åland Islands and 3 regions in SW Finland (see Fig. 1 for a map). Specimens were aggregated based on spatiotemporal information (Dataset S1). As a contemporary reference, we used samples from the Saltvik region in the main Åland Island (Fig. 1), sampled in 2007–2011. For ordination and calculation of F_{ST} values and heterozygosity, we used 40 randomly selected individuals from the total of 4,547 individuals from Saltvik to have a comparable dataset with the other datasets. A similar sample of 40 randomly selected individuals was obtained from the island of Sottunga (a 24-y introduced metapopulation; Fig. 1) out of the total of 319 individuals sampled in 2007–2011. For details of DNA extraction and validation of museum sample genotyping, see SI Appendix.

SNP Selection and Genotyping. The genomic resources for the Glanville fritillary include the published genome (33), a high-density linkage map (59), and two RNA-seq datasets (32, 46), which were used to create a SNP panel using the KASP genotyping platform (LGC Genomics) for large-scale genotyping (Dataset S3). Markers were selected on the basis of previous studies (32, 34, 46, 60), in which candidate genes were associated with flight and dispersal traits or were significantly differentially expressed after an experimental flight treatment. Putatively neutral SNPs were selected from the noncoding regions of the genome (SI Appendix). The remainder of the panel was selected to cover all of the chromosomes ("gap-filling SNP") based on linkage map information (59) (for details of SNP calling, filtering, and validation, see SI Appendix). Following the validation process, 320 SNPs were selected as the final genotyping panel implementing KASP chemistry (LGC Genomics). Museum samples were genotyped for 320 SNPs, whereas contemporary Åland samples were genotyped for a subset of 272 SNPs. Linkage disequilibrium between markers was assessed using GENEPOP v 4.3 (61) with FDR (62) used to correct for multiple testing. At FDR < 0.05, 11 pairs of SNPs showed significant linkage disequilibrium in the Åland and one pair in SW Finnish museum samples (SI Appendix, Table S5). SNP call rates were generally high, >0.97 in contemporary samples and >0.9 in the museum samples. Any SNP with an average call rate <0.7 and any individual with an average call rate <0.8 were excluded from the analysis to eliminate unreliable genotypes (SI Appendix). This filtering resulted in 93 museum specimens and 76 contemporary Åland specimens (Dataset S1; 39 from Saltvik and 37 from Sottunga), genotyped for 222 shared SNPs (Dataset S3).

Genetic Clustering and Diversity. A DAPC (63) using the ADEGENET 2.0 (64) package in R Version 3.2.1 (65) was performed to examine possible genetic clustering in the material. To avoid overfitting, 55 principal components were retained, accounting for ~70% of the total variance. We calculated the F_{ST} value

(66) between each museum specimen and the contemporary Saltvik population, sampled in 2007 ($n = 530$), to estimate the evolutionary distance of each specimen to the reference population (SI Appendix). In addition, we calculated F_{ST} between pairs of aggregated samples using ARLEQUIN v 3.5.1.3 (67) and 10,000 permutations to calculate significance (SI Appendix, Table S1). We calculated sMLH as a surrogate for individual inbreeding (68) using only autosomal markers ($n = 211$). sMLH is calculated for each individual as the total number of heterozygous loci divided by the sum of the observed heterozygosities (across all samples) for the successfully genotyped loci in the focal individual. This takes into account that not all individuals were successfully genotyped for the same set of loci.

Outlier Analysis. To identify outlier loci, we used two different methods using the pooled museum samples from Åland and SW Finland and the Saltvik sample. First, we estimated locus-specific F_{ST} values for SNPs in the candidate genes between the museum samples and the contemporary Saltvik 2007 reference sample (SI Appendix). These values were compared with the posterior distribution of F_{ST} values for the neutral SNPs, and we computed the posterior probability that the F_{ST} was higher for the candidate SNP than for the neutral SNPs. A locus was called an outlier if the posterior probability was higher than 0.95. We used FDR to correct for multiple testing. Second, we tested all 222 SNPs for outliers using BayeScan v 2.1 (69), a Bayesian method that calculates the posterior probability of each locus belonging to a model assuming either selection or no selection, using default parameters.

Allele Frequency Changes in the Outlier Loci. We compared the allele frequencies in the outlier loci between old, well-connected local populations and newly established, isolated populations in the contemporary Åland metapopulation (sampled in 2007–2012 in the Saltvik region, Fig. 1). Old populations were defined as those that had existed for at least three years before sampling, whereas newly established populations were those that had been established by dispersing females in the previous year (the habitat patch had been unoccupied in the year before colonization). Populations were split into the two categories of high or low connectivity (70). The old, well-connected populations consisted of 24 local populations with 903 individuals, and the newly established, isolated populations included 55 populations with 288 individuals. To characterize allele frequency (AF) change due to dispersal and recolonization, we calculated the difference AF(new)-AF(old). The difference AF(new)-AF(old) is weakly correlated with AF(old) (SI Appendix, Fig. S6), but using corrected values gave qualitatively the same results (SI Appendix, Table S6), and we hence retained the uncorrected values for the main analyses. We calculated in the same way the difference in allele frequencies between samples from Sottunga (366 individuals sampled in 2007–2012) and the AF(old) and between the extinct populations in SW Finland and AF(old). Finally, we characterized the allele frequencies of the outlier loci in four regional populations in the northern Baltic region (Fig. 1), two of which have a highly fragmented landscape and two of which have continuous habitat (28, 32). Allele frequencies were obtained from ref. 32. To characterize the allele frequencies in the four regional populations, we ran a principal component analysis (SI Appendix). The second principal component was strongly correlated with the degree of habitat fragmentation (SI Appendix, Table S4). We correlated PC2 with allele frequency change from the contemporary Åland to the introduced Sottunga metapopulation and the extinct SW Finnish populations using PC1 for the latter two samples. PC1 characterizes the average frequencies in the two samples (see SI Appendix for separate analyses for the two populations). We used corrected values for AF(Sottunga)-AF(old) and AF(SW Finland)-AF(old) to remove the effect of (nonsignificant) correlation with AF(old) (see above and SI Appendix) because of very small sample size.

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